

# Prolonged carriage and potential onward transmission of carbapenemase-producing Enterobacteriaceae in Dutch travelers

## Citation for published version (APA):

van Hattem, J. M., Arcilla, M. S., Bootsma, M. C. J., van Genderen, P. J., Goorhuis, A., Grobusch, M. P., Molhoek, N., Oude Lashof, A., Schultsz, C., Stobberingh, E. E., Verbrugh, H. A., de Jong, M. D., Melles, D. C., & Penders, J. (2016). Prolonged carriage and potential onward transmission of carbapenemase-producing Enterobacteriaceae in Dutch travelers. *Future Microbiology*, 11(7), 857-864. <https://doi.org/10.2217/fmb.16.18>

## Document status and date:

Published: 01/07/2016

## DOI:

[10.2217/fmb.16.18](https://doi.org/10.2217/fmb.16.18)

## Document Version:

Publisher's PDF, also known as Version of record

## Document license:

Taverne

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

## RESEARCH ARTICLE

For reprint orders, please contact: [reprints@futuremedicine.com](mailto:reprints@futuremedicine.com)

# Prolonged carriage and potential onward transmission of carbapenemase-producing Enterobacteriaceae in Dutch travelers

Jarne M van Hattem<sup>†,1</sup>, Maris S Arcilla<sup>†,2</sup>, Martin CJ Bootsma<sup>3</sup>, Perry J van Genderen<sup>4</sup>, Abraham Goorhuis<sup>5</sup>, Martin P Grobusch<sup>5</sup>, Nicky Molhoek<sup>6</sup>, Astrid ML Oude Lashof<sup>7</sup>, Constance Schultsz<sup>1</sup>, Ellen E Stobberingh<sup>7</sup>, Henri A Verbrugh<sup>2</sup>, Menno D de Jong<sup>1</sup>, Damian C Melles<sup>2</sup> & John Penders<sup>\*,7</sup>

**Aim:** The aim was to study acquisition and persistence of carbapenemase-producing Enterobacteriaceae (CPE) among travelers. **Materials & methods:** Stools from 2001 travelers and 215 nontraveling household members, collected before and immediately post-travel as well as 1, 3, 6 and 12 months upon return, were screened for CPE. **Results:** Five travelers, all visiting Asia outside the Indian subcontinent, acquired CPE. One traveler persistently carried the same OXA-244 CPE up to 6 months post-travel. Three months after travel, her co-traveling spouse also became positive for this OXA-244 CPE strain, suggesting clonal transmission within this household. **Conclusion:** Acquisition of CPE is not restricted to travelers to the Indian subcontinent and/or to travelers seeking healthcare during travel and can persist up to at least 6 months post-travel.

First draft submitted: 8 October 2015; Accepted for publication: 21 April 2016;  
Published online: 30 June 2016

The Indian subcontinent has been identified as an important reservoir of carbapenemase-producing Enterobacteriaceae (CPE). Indeed, acquisition of CPE during travel to India has recently been reported, illustrating the risk of further global spread by travelers to this CPE-endemic region [1]. However, acquisition of CPE in healthy travelers to Asian regions other than the Indian subcontinent is suggested but has not been reported from prospective studies thus far. There are a number of prospective studies on acquisition of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae (ESBL-E) and CPE in healthy travelers [1–10]. Except for the above-mentioned study on CPE acquisition in travelers to India [1], none of these studies identified subjects with CPE acquisition during travel.

Within the context of a large-scale prospective cohort of healthy travelers, the COMBAT study, we aimed to determine CPE acquisition, persistence of colonization and potential onward transmission.

## Materials & methods

### • Design & data collection

As part of the COMBAT study (ClinicalTrials.gov identifier: NCT01676974), a multicenter longitudinal cohort of healthy travelers ( $n = 2001$ ) and their nontraveling household members ( $n = 215$ )

## KEYWORDS

- carbapenemases
- Enterobacteriaceae
- transmission • travel

<sup>1</sup>Academic Medical Center, Department of Medical Microbiology, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

<sup>2</sup>Erasmus University Medical Center, Department of Medical Microbiology & Infectious Diseases, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

<sup>3</sup>Utrecht University, Department of Mathematics, Faculty of Science, Budapestlaan 6, 3584 CD Utrecht, The Netherlands

<sup>4</sup>Havenziekenhuis – Institute for Tropical Diseases, Department of Internal Medicine, Haringvliet 2, 3011 TD Rotterdam, The Netherlands

<sup>5</sup>Academic Medical Center, Center for Tropical Medicine & Travel Medicine, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

<sup>6</sup>Havenziekenhuis – Travel Clinic Harbour Hospital, Haringvliet 2, 3011 TD Rotterdam, The Netherlands

<sup>7</sup>Maastricht University Medical Center, NUTRIM School for Nutrition & Translational Research in Metabolism and Caphri School for Public Health & Primary Care, Department of Medical Microbiology, PO 5800, 6202 AZ Maastricht, The Netherlands

\*Author for correspondence: Tel.: +31 43 3875134; Fax: +31 43 3884128; [j.penders@maastrichtuniversity.nl](mailto:j.penders@maastrichtuniversity.nl)

<sup>†</sup>Authors contributed equally

was followed. Participants were recruited within a period of 1 year, from November 2012 until November 2013 at the outpatient clinics run by the Academic Medical Center (Amsterdam, The Netherlands), Havenziekenhuis (Rotterdam, The Netherlands) and Maastricht University Medical Center/Public Health Service South Limburg (Maastricht, The Netherlands). Adult ( $\geq 18$  years) travelers visiting one of the above-stated travel clinics, traveling abroad for a minimum of 1 week to a maximum of 3 months were eligible for participation. Minors ( $< 18$  years) and incapacitated subjects are excluded from this study. No restriction was applied with respect to travel destination.

Fecal samples (Fecal Swab<sup>®</sup>; Copan, Brescia, Italy) were collected before, immediately after (within 1–2 weeks) and 1 month after return from all participants. Follow-up fecal samples were collected 3, 6 and 12 months after return from travelers (and their household members) who acquired extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae (ESBL-E) and/or CPE. Ethical approval was obtained by the Medical Ethical Committee of Maastricht University Medical Center (study number: METC 12-4-093). A full description of the study design has been published elsewhere [11].

#### • Microbiological methods

Fecal samples were enriched overnight at 35°C in TSB with vancomycin (50 mg/l) to prevent overgrowth of Gram-positive bacteria [12]. Ten microliters were inoculated on chromID<sup>®</sup> ESBL agar (bioMérieux, Marcy l'Etoile, France) and incubated overnight at 35°C. Screening with this cefpodoxime-containing agar aims at maximum detection of ESBL-E. However some CPE, such as those producing OXA-48-like  $\beta$ -lactamases not co-producing an ESBL, remain susceptible and can be missed using this agar [13]. To see what the proportion of ESBL-negative, OXA-48-positive CPE was in our cohort, a substudy was held from August until November 2013. In total, 500 consecutive post-travel samples were additionally screened for OXA-48 CPE using chromID OXA-48 agar. All colonies of different morphologies growing on any of these two agars were characterized to the species level using MALDI-TOF (Bruker, London, UK). Minimum inhibitory concentrations (MICs) were measured for all Enterobacteriaceae by the use of the Vitek 2 system (bioMérieux, Marcy l'Etoile, France).

Phenotypic confirmation of ESBL production was performed by the combination disk diffusion

test according to current national Dutch guidelines [14]. Isolates with MICs for imipenem  $> 1$  mg/l or for meropenem  $> 0.25$  mg/l, confirmed with E-test (bioMérieux), were considered possible carbapenemase producers [14] and genotypically characterized. In case a CPE was cultured from the post-travel sample (either from the ESBL or OXA-48 agar) specific PCR for the detected carbapenemase gene was performed on fecal metagenomic DNA of the pretravel sample. All CPE suspected isolates were screened for the presence of multiple classes of ESBL and carbapenemase genes using microarray (Identibac<sup>®</sup> AMR08; Alere Technologies GmbH, Jena, Germany) [15,16]. Targeted PCR and DNA sequencing of the PCR-amplicons was performed with primers as described previously [15,17–23] and in-house primers. The underlined nucleotide from the IMI primer 5'-CAAAGCAAATGAACGATTTC-3' was modified from [23]. DNA was extracted as described by Anjum *et al.* [15]. The lysate containing the crude DNA was used for biotin labeling and PCR.

All acquired carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from post-travel samples were further analyzed with multilocus sequence typing (MLST) [24,25] and sequence types (ST) were assigned by querying the respective MLST databases for *E. coli* [26] and *K. pneumoniae* [27].

To determine persistence of carriage and clonal transmission, amplification fragment length polymorphism (AFLP) [28] was assayed for all consecutive CPE isolates of the travelers and their household members.

## Results

More than half of all included travelers visited destinations in Asia ( $n = 1016/2001$ , 50.8%), while Africa was visited by 633 travelers (31.6%). America (mainly South and Central America), Europe and Oceania were visited by 326 (16.3%), 21 (1.0%) and 5 (0.2%) travelers, respectively.

Prior to travel, one participant was carrying an OXA-48-producing *E. coli* and as such excluded from subsequent analysis. This 47-year-old healthy male subject had no travel history in the previous 12 months, but had been admitted to a Dutch hospital and treated with azithromycin within the preceding 3 months.

In five travelers, CPE was detected in specimens collected immediately upon return. Specific PCR for the detected carbapenemase-encoding

gene on fecal metagenomic DNA of the pretravel sample was negative in all five cases. Subject- and travel-related characteristics of the travelers who acquired CPE during travel, as well as the molecular characteristics and dynamics over time of acquired CPE isolates, are summarized in **Table 1**.

Out of the five travelers who acquired CPE, none had sought medical care during their travel, all but one (subject 3) reported diarrhea during travel and one traveler (subject 5) had used broad-spectrum antibiotics (**Table 1**). This traveler used an oral drug called *Disento* for complaints of watery diarrhea during travel (a mix of quinoline, aminoglycoside, nitrofurantoin and sulfonamide).

Out of the acquired CPEs, three isolates were *E. coli* of various sequence types and encoding different carbapenemase genes, one was an *bla*<sub>OXA-48</sub>-encoding ST363 *K. pneumoniae* and one an *E. cloacae* complex harboring an *bla*<sub>IMI-2</sub> gene (**Table 1**).

Similar to the previous report by Ruppé *et al.* [1], carriage of acquired CPE was of limited duration in three of our travelers, in whom CPE could not be detected 1 month after return from travel (**Table 1**). However, in one traveler, CPE carriage persisted at least 1 month and in another traveler (subject 2a), an OXA-244-producing *E. coli* isolate persisted for at least 6 months after return, as evidenced by similar AFLP patterns of isolates in follow-up specimens (**Figure 1**). Three months after travel, an OXA-244 *E. coli* isolate with a similar AFLP pattern was isolated from a fecal sample collected from her spouse and travel companion (subject 2b; **Table 1 & Figure 1**). As all other fecal specimens from this subject were CPE negative, this strongly suggests post-travel acquisition of the same bacterium through transmission from his wife.

## Discussion

Our report underscores that CPE are indeed acquired during travel by healthy travelers in the absence of exposure to local healthcare during travel. Importantly, our observations also indicate that a risk of such acquisition during travel is not limited to travelers to the Indian subcontinent. In fact, none of 119 travelers in our cohort who traveled to India had acquired CPE. Instead, CPE acquisition was observed in five study participants who traveled to Europe and countries in south-eastern, eastern and western Asian, including two out of 23 visitors to Myanmar.

The low prevalence of CPE in the pretravel samples from our study (one of 2001 subjects was positive for CPE) is consistent with the very low background carriage of CPE in the Dutch community as described previously. In two studies on the prevalence and molecular characteristics of ESBL-E in the Dutch community, conducted in 2010 and 2011 and including 720 and 1033 subjects, respectively, no CPE or carbapenemase-encoding genes were found [29,30]. Moreover, the prevalence of carbapenemase resistance in clinical isolates in The Netherlands was only 0.01% for *E. coli* and 0.15% for *K. pneumoniae* in 2013–2014 [31].

One traveler to Myanmar acquired an *E. cloacae* complex isolate harboring an *bla*<sub>IMI-2</sub> gene. The IMI  $\beta$ -lactamases are a relatively uncommon group of carbapenemases. They are sporadically found in clinical isolates and environmental isolates from rivers in the USA [20] and in clinical isolates from China [32] and France [33]. Acquisition of IMI carbapenemases in travelers has not been described yet. This acquisition shows that travel might not only play a role in the spread of more common OXA-48-like and NDM-carbapenemases, but also of rarer plasmid-encoded carbapenemases such as IMI-2.

Another traveler to Myanmar acquired an ST162 *E. coli* isolate carrying an *bla*<sub>NDM-7</sub> gene. Although data on the prevalence of antimicrobial resistance in Myanmar are very limited, it is interesting to note that the first NDM-7 *E. coli* was recovered in France from urine of a female patient who also traveled to Myanmar [34]. Concordantly with the isolate retrieved in our study, this isolate also harbored a *bla*<sub>CTX-M-15</sub> ESBL gene, but belonged to a different sequence type (ST167).

A second *bla*<sub>NDM</sub> gene was acquired by a traveler when traveling throughout south-eastern and eastern Asia. NDM-producers are considered to be endemic in India and Pakistan [35], but likely have spread from the Indian subcontinent to neighboring countries and throughout Asia as reflected by the acquisition of *bla*<sub>NDM</sub> genes in the two travelers that traveled outside these countries and confirmed by publications on the emergence of NDM-producing Enterobacteriaceae in Thailand [36].

One of the 270 travelers that visited Indonesia acquired a CPE: an OXA-244 positive *E. coli* isolate belonging to ST38. OXA-244 is an OXA-48-like  $\beta$ -lactamase that exhibits weak carbapenemase activity and which differs by a single amino acid substitution from classical

Table 1. Characteristics of travelers that acquired carbapenemase-producing Enterobacteriaceae during travel, characteristics of journeys made, characteristics of acquired carbapenemase-producing Enterobacteriaceae isolates and dynamics of acquired carbapenemase-producing Enterobacteriaceae over time.																
Characteristics of travelers				Characteristics of journeys			Characteristics of acquired CPE				Dynamics of CPE over time					
Traveler	Age	Sex	Chronic diseases	Countries visited	Duration (days)	Period	Purpose of visit	Species	MLST	ESBL gene(s)	Before travel	On return	1 month after travel	3 months after travel	6 months after travel	12 months after travel
1	64	F	Type II diabetes	Myanmar	16	July 2013	Maritime study trip	<i>Enterobacter cloacae</i> complex	ND	None	– <sup>†</sup>	IMI-2 <sup>††</sup>	– <sup>†</sup>	– <sup>†</sup>	– <sup>†</sup>	– <sup>†</sup>
2a	58	F		Indonesia	22	August 2013	Backpack holiday	<i>Escherichia coli</i>	ST38	CTX-M-14	– <sup>†</sup>	OXA-244 <sup>††</sup>	OXA-244 <sup>††</sup>	OXA-244 <sup>††</sup>	OXA-244 <sup>§§</sup>	– <sup>§</sup>
2b	59	M	'Cardiac arrhythmia'	Indonesia	22	August 2013	Backpack holiday	<i>E. coli</i>	ST38	CTX-M-14	– <sup>†</sup>	– <sup>†</sup>	– <sup>†</sup>	OXA-244 <sup>††</sup>	– <sup>§</sup>	– <sup>§</sup>
3	41	M		Turkey, Greece	14	September 2013	Active/backpack holiday	<i>Klebsiella pneumoniae</i>	ST363	None	– <sup>†</sup>	OXA-48 <sup>§§</sup>	– <sup>§</sup>	– <sup>§</sup>	– <sup>§</sup>	– <sup>§</sup>
4	37	F	Asthma, hypothyroidism	China, Thailand, Vietnam, Japan, Hong Kong and Singapore	22	October 2013	Luxury/wellness holiday	<i>E. coli</i>	ST2914	CTX-M-15 and CTX-M-55	– <sup>†</sup>	NDM-1/2 <sup>††</sup>	NDM-1/2 <sup>††</sup>	– <sup>†</sup>	– <sup>†</sup>	– <sup>†</sup>
5	64	F	Seborrheic eczema	Myanmar	22	October 2013	Active/backpack holiday	<i>E. coli</i>	ST162	CTX-M-15	– <sup>†</sup>	NDM-7 <sup>††</sup>	– <sup>†</sup>	– <sup>†</sup>	– <sup>†</sup>	– <sup>†</sup>
Travelers 2a and 2b belong to the same household. None of the travelers had sought medical care during their travel, all but one (subject 3) reported diarrhea during travel and one traveler (subject 5) had used broad-spectrum antibiotics.																
<sup>†</sup> Screening with chromID <sup>®</sup> ESBL agar.																
<sup>††</sup> Isolated from chromID ESBL agar.																
<sup>§</sup> Screening with both chromID ESBL agar and chromID OXA-48 agar.																
<sup>§§</sup> Isolated from both chromID ESBL and chromID OXA-48 agar.																
–: No CPE isolated; CPE: Carbapenemase-producing Enterobacteriaceae; ESBL: Extended-spectrum β-lactamase-producing Enterobacteriaceae; ND: Not determined; MLST: Multilocus sequence typing.																



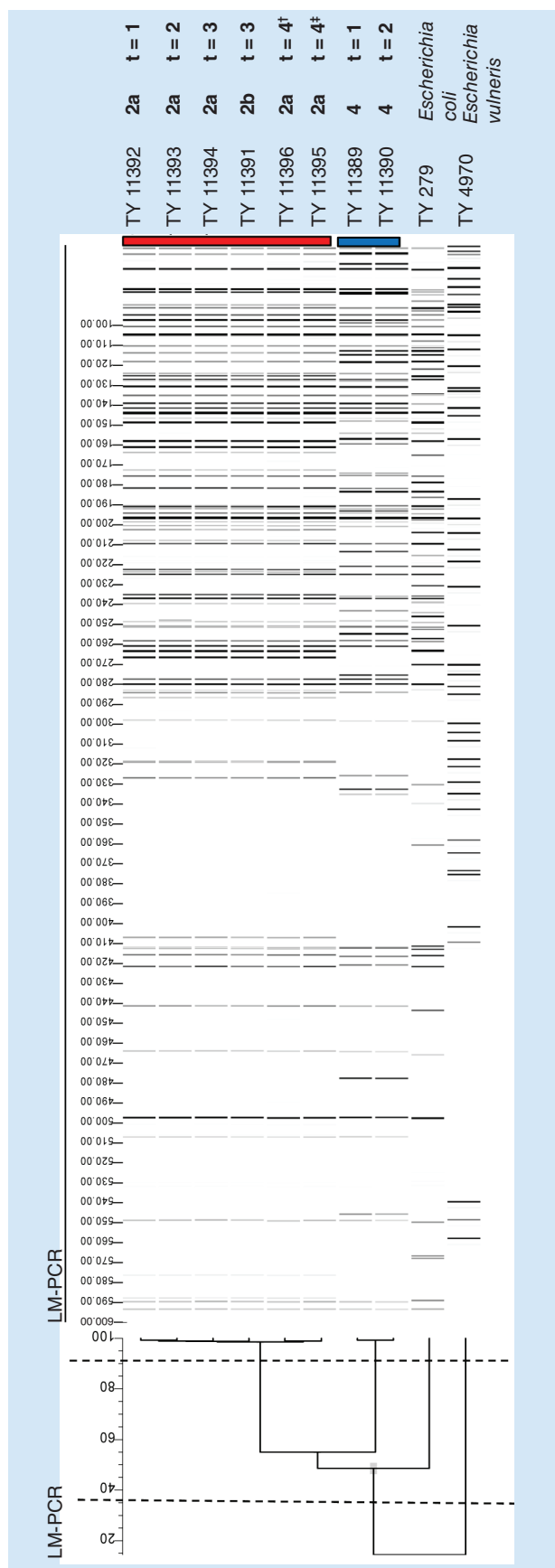
OXA-48 [37]. ST38-type *E. coli* isolates harboring a classical *bla*<sub>OXA-48</sub> gene have previously been recovered from Lebanon, Egypt, Turkey, Switzerland and France [38–41]. More recently, the OXA-48-like variant OXA-244 was also identified in an ST38 *E. coli* isolated from a hospitalized patient in France without any travel history [42]. To our knowledge, there are no reports of OXA-48-like harboring Enterobacteriaceae from Indonesia.

An OXA-48 harboring *K. pneumoniae* isolate was acquired by a traveler that visited Turkey and Greece. For many years, almost all the reports of OXA-48-producers remained from patients hospitalized in Turkey or from patients with a link to Turkey [21]. The endemicity of OXA-48 in this country has most likely resulted to the acquisition in this traveler, as Greece is known as an important reservoir for KPC, but not for OXA-48 [35].

Household transmission of travel-acquired CPE between healthy subjects 2a and 2b was strongly suspected, although the possibility cannot be fully excluded that subject 2b also acquired CPE during travel but that this remained undetected in initial specimens. Possible household transmission from a documented carbapenemase-producing *K. pneumoniae* carrier – a female with amyotrophic lateral sclerosis that required mechanical ventilation and had been hospitalized in a tertiary hospital in the Tel Aviv area – to her spouse has previously been reported [43]. Another paper describes possible vertical or horizontal transmission of an NDM-1-producing *Enterobacter cloacae* in an Australian newborn that did not travel overseas [44].

The COMBAT-study aimed at maximum detection of ESBL-E, which might have led to an underestimation of acquisition of OXA-48-like  $\beta$ -lactamase producers. In our subset of 500 travelers, one additional OXA-48 acquisition was found (subject 3). This indicates that likely only a few additional OXA-48 acquisitions would have been detected when all 2001 subjects would have been screened with OXA-48 agar.

Literature on antimicrobial resistance in resource-limited settings is infrequent thereby hampering the comparison between CPEs acquired by travelers and the local prevalence and molecular characteristics of CPEs at the travel destination. However, travelers may act as a sentinel for emerging local resistance in developing countries like Indonesia and Myanmar as illustrated in this paper.



**Figure 1. Genetic relatedness among travel-acquired carbapenemase-producing *Escherichia coli*.** Red and blue bars indicate isolates with identical amplification fragment length polymorphism pattern. Cut-off for identical strains is set at 90% as indicated by the right dashed line.

<sup>†</sup>Isolated from chromID® ESBL agar.

<sup>‡</sup>Isolated from chromID OXA-48 agar.

2a: Subject 2a; 2b: Subject 2b; 4: Subject 4; t = 1: Within 1 week after travel; t = 2: 1 month after travel; t = 3: 3 months after travel; t = 4: 6 months after travel.

### Conclusion & future perspective

In conclusion, the risk of acquisition of CPE during travel is not restricted to travelers to the Indian subcontinent and/or to travelers seeking healthcare during travel and carriage of travel-acquired CPE can persist up to at least 6 months after return from travel. Prolonged carriage obviously increases the risk of onward transmission and further spread of CPE. These observations deserve consideration by healthcare providers and public health professionals worldwide. Particularly in countries with low-level prevalence of CPE, screening for CPE in patients who are admitted to healthcare facilities should be considered, not only after recent travel, but even several months after returning from high-risk countries.

### Acknowledgements

The authors would like to thank Muna Anjum and Roderick Card from the Animal Health and Veterinary

Laboratories Agency (AHVLA), Weybridge, UK, for their help with the microarray.

### Financial & competing interests disclosure

This study was funded by The Netherlands Organization for Health, Research and Development (ZonMw) programme Priority Medicine Antimicrobial Resistance (project number: 50-51700-98-120). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

### Open access

This work is licensed under the Attribution-NonCommercial-NoDerivatives 4.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

## EXECUTIVE SUMMARY

### Background

- Acquisition of carbapenemase-producing Enterobacteriaceae (CPE) in healthy travelers without local healthcare contact during travel has recently been described for travelers to India.

### Results

- This study reports acquisition of CPE in five travelers to Asia that did not travel across the Indian subcontinent.
- None of these travelers had sought medical care during their travel, all but one reported diarrhea during travel and one traveler had used broad-spectrum antibiotics.
- Persistence of colonization up to at least 6 months after return from travel was found for one traveler.
- In one of the CPE-positive travelers evidence was found for clonal transmission of OXA-244 *Escherichia coli* to her spouse.

### Conclusion

- Acquisition of CPE during travel is neither restricted to travelers to the Indian subcontinent nor to travelers seeking healthcare during travel.
- Screening for CPE in patients who are admitted to healthcare facilities should be considered, not only after recent travel, but even several months after returning from high-risk countries.

### References

Papers of special note have been highlighted as:  
• of interest; •• of considerable interest.

- Ruppe E, Armand-Lefevre L, Estellat C *et al.* Acquisition of carbapenemase-producing Enterobacteriaceae by healthy travellers to India, France, February 2012 to March 2013. *Euro Surveill.* 19(14), pii: 20768 (2014).
- Kennedy K, Collignon P. Colonisation with *Escherichia coli* resistant to “critically important” antibiotics: a high risk for international travellers. *Eur. J. Clin. Microbiol. Infect. Dis.* 29(12), 1501–1506 (2010).
- Tangden T, Cars O, Melhus A *et al.* Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob. Agents Chemother.* 54(9), 3564–3568 (2010).
- Weisenberg SA, Mediavilla JR, Chen L *et al.* Extended spectrum beta-lactamase-producing Enterobacteriaceae in international travelers and non-travelers in New York City. *PLoS ONE* 7(9), e45141 (2012).

- 5 Paltansing S, Vlot JA, Kraakman MEM *et al.* Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae among travelers from The Netherlands. *Emerg. Infect. Dis.* 19(8), 1206–1213 (2013).
- 6 Ostholm-Balkhed A, Tarnberg M, Nilsson M *et al.* Travel-associated faecal colonization with ESBL-producing Enterobacteriaceae: incidence and risk factors. *J. Antimicrob. Chemother.* 68(9), 2144–2153 (2013).
- 7 Kuenzli E, Jaeger VK, Frei R *et al.* High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Swiss travellers to south Asia – a prospective observational multicenter cohort study looking at epidemiology, microbiology and risk factors. *BMC Infect. Dis.* 14, 528 (2014).
- 8 Lubbert C, Straube L, Stein C *et al.* Colonization with extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacteriaceae in international travelers returning to Germany. *Int. J. Med. Microbiol.* 305(1), 148–156 (2015).
- 9 Kantele A, Laaveri T, Mero S *et al.* Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing Enterobacteriaceae. *Clin. Infect. Dis.* 60(6), 837–846 (2015).
- **One of the largest prospective studies on the acquisition of extended-spectrum  $\beta$ -lactamase- and carbapenemase-producing Enterobacteriaceae in healthy travelers. Out of the 430 travelers, none acquired CPE.**
- 10 Angelin M, Forsell J, Granlund M *et al.* Risk factors for colonization with extended-spectrum beta-lactamase producing Enterobacteriaceae in healthcare students on clinical assignment abroad: a prospective study. *Travel Med. Infect. Dis.* 13(3), 223–229 (2015).
- 11 Arcilla MS, Van Hattem JM, Bootsma MC *et al.* The Carriage of Multiresistant Bacteria after Travel (COMBAT) prospective cohort study: methodology and design. *BMC Public Health* 14, 410 (2014).
- **Describes the population and design of the COMBAT study.**
- 12 Murk JL, Heddema ER, Hess DL *et al.* Enrichment broth improved detection of extended-spectrum-beta-lactamase-producing bacteria in throat and rectal surveillance cultures of samples from patients in intensive care units. *J. Clin. Microbiol.* 47(6), 1885–1887 (2009).
- 13 Carrer A, Fortineau N, Nordmann P. Use of ChromID extended-spectrum beta-lactamase medium for detecting carbapenemase-producing Enterobacteriaceae. *J. Clin. Microbiol.* 48(5), 1913–1914 (2010).
- 14 Bernards AT, Bonten MJ, Cohen Stuart J *et al.* NVMM Guideline Laboratory detection of highly resistant microorganisms (HRMO) (2) 25–46 (2012). [www.nvmm.nl/richtlijnen/hrmo-laboratory](http://www.nvmm.nl/richtlijnen/hrmo-laboratory)
- 15 Anjum MF, Choudhary S, Morrison V *et al.* Identifying antimicrobial resistance genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain. *J. Antimicrob. Chemother.* 66(3), 550–559 (2011)
- 16 Card R, Zhang J, Das P *et al.* Evaluation of an expanded microarray for detecting antibiotic resistance genes in a broad range of gram-negative bacterial pathogens. *Antimicrob. Agents Chemother.* 57(1), 458–465 (2013).
- 17 Pitout JD, Hossain A, Hanson ND. Phenotypic and molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella* spp. *J. Clin. Microbiol.* 42(12), 5715–5721 (2004).
- 18 Paauw A, Fluit AC, Verhoef J *et al.* *Enterobacter cloacae* outbreak and emergence of quinolone resistance gene in Dutch hospital. *Emerg. Infect. Dis.* 12(5), 807–812 (2006).
- 19 Eckert C, Gautier V, Saladin-Allard M *et al.* Dissemination of CTX-M-type beta-lactamases among clinical isolates of Enterobacteriaceae in Paris, France. *Antimicrob. Agents Chemother.* 48(4), 1249–1255 (2004).
- 20 Aubron C, Poirel L, Ash RJ *et al.* Carbapenemase-producing Enterobacteriaceae U.S. rivers. *Emerg. Infect. Dis.* 11(2), 260–264 (2005).
- 21 Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J. Antimicrob. Chemother.* 67(7), 1597–1606 (2012).
- 22 Poirel L, Dortet L, Bernabeu S *et al.* Genetic features of blaNDM-1-positive Enterobacteriaceae. *Antimicrob. Agents Chemother.* 55(11), 5403–5407 (2011).
- 23 Pasteran F, Mendez T, Guerriero L *et al.* Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae. *J. Clin. Microbiol.* 47(6), 1631–1639 (2009).
- 24 Wirth T, Falush D, Lan R *et al.* Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* 60(5), 1136–1151 (2006).
- 25 Diancourt L, Passet V, Verhoef J *et al.* Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J. Clin. Microbiol.* 43(8), 4178–4182 (2005).
- 26 MLST website hosted at the Warwick University, UK. <http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>
- 27 MLST website hosted at Institut Pasteur, France. <http://bigsd.web.pasteur.fr/klebsiella/>
- 28 Savelkoul PH, Aarts HJ, De Haas J *et al.* Amplified-fragment length polymorphism analysis: the state of an art. *J. Clin. Microbiol.* 37(10), 3083–3091 (1999).
- 29 Van Hoek AH, Schouls L, Van Santen MG, Florijn A, De Greeff SC, Van Duikeren E. Molecular characteristics of extended-spectrum cephalosporin-resistant Enterobacteriaceae from humans in the community. *PLoS ONE* 10(6), e0129085 (2015).
- 30 Reuland EA, Overdevest IT, Al Naiemi N *et al.* High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. *Clin. Microbiol. Infect.* 19(6), 542–549 (2013).
- **Describes the absence of CPE in a population of 720 Dutch community patients, highlighting the very low carriage rate of CPE in The Netherlands.**
- 31 NethMap. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in The Netherlands in 2014. [www.swab.nl/swab/cms3.nsf/uploads/](http://www.swab.nl/swab/cms3.nsf/uploads/)
- 32 Huang L, Wang X, Feng Y *et al.* First identification of an IMI-1 carbapenemase-producing colistin-resistant *Enterobacter cloacae* in China. *Ann. Clin. Microbiol. Antimicrob.* 14(1), 51 (2015).
- 33 Dupont H, Gaillot O, Goetgheluck AS *et al.* Molecular characterization of carbapenem-non-susceptible Enterobacterial isolates collected during a prospective interregional survey in France and susceptibility to the novel ceftazidime-avibactam and aztreonam-avibactam combinations. *Antimicrob. Agents Chemother.* 60(1), 215–221 (2015).
- 34 Cuzon G, Bonnin RA, Nordmann P. First identification of novel NDM carbapenemase, NDM-7, in *Escherichia coli* in France. *PLoS ONE* 8(4), e61322 (2013).
- 35 Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin. Microbiol. Infect.* 20(9), 821–830 (2014).
- 36 Rimrang B, Chanawong A, Lulitanond A *et al.* Emergence of NDM-1- and IMP-14a-producing Enterobacteriaceae in Thailand. *J. Antimicrob. Chemother.* 67(11), 2626–2630 (2012).
- 37 Oteo J, Hernandez JM, Espasa M *et al.* Emergence of OXA-48-producing *Klebsiella*



- pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. *J. Antimicrob. Chemother.* 68(2), 317–321 (2013).
- 38 Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 beta-lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. *Euro Surveill.* 18(31), pii:20549 (2013).
- 39 Dimou V, Dhanji H, Pike R, Livermore DM, Woodford N. Characterization of Enterobacteriaceae producing OXA-48-like carbapenemases in the UK. *J. Antimicrob. Chemother.* 67(7), 1660–1665 (2012).
- 40 Beyrouthy R, Robin F, Dabboussi F *et al.* Carbapenemase and virulence factors of Enterobacteriaceae in North Lebanon between 2008 and 2012: evolution via endemic spread of OXA-48. *J. Antimicrob. Chemother.* 69(10), 2699–2705 (2014).
- 41 Zurfluh K, Nuesch-Inderbinen MT, Poirel L *et al.* Emergence of *Escherichia coli* producing OXA-48 beta-lactamase in the community in Switzerland. *Antimicrob. Resist. Infect. Control* 4, 9 (2015).
- 42 Potron A, Poirel L, Dortet L *et al.* Characterisation of OXA-244, a chromosomally-encoded OXA-48-like beta-lactamase from *Escherichia coli*. *Int. J. Antimicrob. Agents* 47(1), 102–103 (2015).
- 43 Gottesman T, Agmon O, Shwartz O *et al.* Household transmission of carbapenemase-producing *Klebsiella pneumoniae*. *Emerg. Infect. Dis.* 14(5), 859–860 (2008).
- **Case report of household transmission of carbapenemase-producing *Klebsiella pneumoniae* of nosocomial origin in Israel.**
- 44 Blyth CC, Pereira L, Goire N. New Delhi metallo-beta-lactamase-producing enterobacteriaceae in an Australian child who had not traveled overseas. *Med. J. Aust.* 200(7), 386 (2014).